expression. The Applicant believes that these claims are allowable and cover the same subject matter purportedly covered by U.S. Patent No. 5,272,071 to Chappel ("'071 Chappel patent") of record, and that an interference should be declared.

The remaining claims, (new Claims 107-119) cover methods for the large-scale production of proteins in cell culture involving targeting a gene in a mammalian host cell and transferring the targeted recombined gene to a mammalian continuous cell line which is cultured for large scale production of the target gene product. The Applicant believes that Claims 107-119 are separately patentable and should not be part of the interference contest proposed with the Chappel '071 patent.

The new claims are fully supported by the instant specification and claims as originally filed. No new matter is introduced by these amendments.

In particular, support for claim 106, covering gene activated mammalian cells in which the target gene is EPO (erythropoietin), the host cell is a human 293 embryonal kidney cell, and the regulatory sequence is the CMV promoter/enhancer, can be found in the example at pages 15-20 of the specification.

The method of Claims 107-119, involving targeting an amplifiable gene and/or a regulatory sequence into a resident gene in a mammalian host cell, and transferring the targeted gene to a continuous mammalian cell line for large scale protein production in culture is described in the "Summary of the Invention," p. 3, lines 11-26; "Description of Specific

Embodiments," pp. 4-12; and in the working examples described on pp. 12-19.

The human and primary cells specified in Claims 111-113 are described in the specification at p. 4, line 30 to p. 5, line 10; the mammalian target genes specified in dependent Claims 114-115 are described in the specification at p. 5, line 24 to p. 6, line 2; the regulatory sequences specified in Claims 116-117 are described in the specification at p. 7, lines 1-35 and exemplified using the CMV promoter/enhancer at pp. 15-19; the amplifiable genes specified in Claim 118 are described in the specification at p. 5, lines 11-23 and used in the working examples at pp. 12-19; and the continuous cell lines specified in Claim 119 are described in the specification at p. 11, lines 10-20.

1. CLAIMS 105 AND 106, COVERING GENE ACTIVATION, ARE PATENTABLE

The specification is objected to and Claim 105, attached hereto as Appendix A, covering mammalian cells with activated target genes, is rejected under 35 U.S.C. § 112, first paragraph, as being non-enabled and lacking a written description. Also, Claim 105 is rejected under 35 U.S.C. § 102(e) in view of U.S. Patent No. 5,272,071 to Chappel ("Chappel '071 patent"). These objections and rejections should be withdrawn for reasons detailed below.

1.1. The Subject Matter Of Claims 105 And 106 Is Enabled Under Section 112, First Paragraph

The Examiner asserts that the specification fails to disclose how to express a gene product encoded by a normally

E

transcriptionally silent target gene. According to the Examiner, two embodiments in the specification, *i.e.*, the expression of EPO in human embryonic kidney cells and the expression of tPA in human primary diploid fibroblasts, deal with cell lines which constitutively express the target gene.

The Examiner further states that wherein the gene is normally transcriptionally silent, transcriptional silence of genes is known in the art to be due to a wide variety of factors, such as gene silencers, mutations in coding regions or regulatory regions of genes or lack of transactivating factors needed to induce transcription. The Examiner asserts that the specification fails to identify those particular genes wherein the "gene is normally transcriptionally silent" and to teach one of ordinary skill methods to overcome transcriptional silence in those instances. The Examiner contends that in view of the lack of direction or guidance in the specification regarding which genes are transcriptionally silent and methods of overcoming the transcriptional silence, it would require undue experimentation by one of ordinary skill to determine why a particular gene is transcriptionally silent and to determine the method to overcome the silence.

Applicant traverses this Section 112, first paragraph, enablement objection and rejection. Contrary to the assertion by the Examiner, Applicant's embodiment relating to the expression of the EPO gene in a human 293 embryonal kidney cell is an embodiment of a mammalian host cell that expresses a gene product encoded by a normally transcriptionally silent target gene! Applicant submits herewith a Rule 132 Declaration by Dr. Mitchell Finer that

E

demonstrates that the EPO gene is normally transcriptionally silent in human 293 embryonal kidney cells (¶ 11, Finer Declaration).[1] Thus, Applicant does teach in the specification the activation of a normally transcriptionally silent gene.

Further, contrary to the assertion by the Examiner, Claim 105 does not require one to determine why a particular target gene is transcriptionally silent so that one can predict which genes would be expected to be transcriptionally active upon insertion of a heterologous promoter via homologous recombination. Also, contrary to the assertion by the Examiner, Claim 105 does not require that one be able to express every transcriptionally silent gene.

First, all that is required by Claim 105 is that one determine whether or not a target gene is transcriptionally silent. One skilled in the art could, without undue experimentation, determine whether or not a particular gene is transcriptionally silent. It would be routine for one skilled in the art to determine whether or not a particular gene is transcriptionally silent because one only need perform a Northern blot assay or a reverse transcriptase-PCR (RT-PCR) analysis, each of which is a routine assay for mRNA. In fact, Dr. Finer carried out a RT-PCR analysis for RNA isolated from human 293 embryonal kidney cells (¶¶ 8-10, Finer Declaration).

---

[1] With respect to the embodiment that the Examiner asserts that human diploid fibroblasts are known to constitutively express tPA, Applicant is not so aware. Thus, Applicant requests that the Examiner provide a reference to support such assertion or a Rule 107(b) declaration stating the same.

E

Second, once one determines that a particular target gene is normally transcriptionally silent, Claim 105 covers only those host cells wherein the targeted host cell "expresses the target gene product." Thus, Claim 105 contains a functional limitation to exclude those host cells that do not express the target gene.

The law is settled that one can draft a claim with a functional limitation so as to exclude those embodiments that do not provide a desired benefit. *In re Halleck*, 164 USPQ 647 (CCPA 1970), *In re Boller*, 141 USPQ 740 (CCPA 1964) and *In re Fuetterer*, 138 USPQ 217 (CCPA 1963).

Finally, to determine whether or not a particular target gene would be transcriptionally activated by the insertion of a heterologous promoter only requires that one construct the host cell of Claim 105 and then screen for the target gene product. This is a routine procedure. If the target gene product is present, then the host cell is within the scope of Claim 105. If the target gene product is not present, then the host cell is not within the scope of Claim 105. One need not, as implied by the Examiner, be able to predict this result, and Applicant is not claiming every transcriptionally silent gene. There may exist transcriptionally silent genes that cannot be expressed by Applicant's invention.

In summary, Claim 105 is enabled because one skilled in the art could routinely determine whether a particular target gene is transcriptionally silent in a host cell and determine whether that transcriptionally silent gene is activated by an inserted heterologous regulatory sequence so

E

that the host cell expresses the target gene product. Furthermore, Applicant's specification does, in fact, exemplify how to express a gene product encoded by a normally transcriptionally silent target gene.

Accordingly, Applicant requests that this Section 112, first paragraph, enablement objection and rejection be withdrawn.

In addition, the subject matter of Claim 106 is enabled because it covers a human 293 embryonal kidney cell that expresses EPO wherein the genome of the kidney cell has inserted therein the enhancer and promoter of human CMV operatively associated with the EPO gene, which, as acknowledged by the Examiner, is exemplified in the specification.

1.2. The Subject Matter of Claims 105 And 106 Is Described In The Instant Specification

The Examiner asserts that the specification fails to provide a written description for the claim language of Claim 105. Specifically, the Examiner states that the specification discloses, at page 5, lines 5-6, that "the gene of interest may or may not be expressed" and does not provide either literal or inherent support for the phrase "normally transcriptionally silent target gene". According to the Examiner, it is known in the art that gene expression involves protein production and, therefore, both transcription and translation. Thus, the Examiner alleges that when the "gene of interest may or may not be expressed" the lack of expression may be related to the translational process and

unrelated to transcriptional silence. Therefore, the Examiner concludes that in view of the multiple reasons for lack of gene expression, one of ordinary skill does not immediately envision "transcriptional silence" from the phrase "the gene of interest may or may not be expressed".

Applicant traverses this Section 112, first paragraph, written description objection and rejection. The law is settled that compliance with the written description requirement does not require an applicant to describe exactly the subject matter claimed; rather, the description must clearly allow a person of ordinary skill in the art to recognize that he or she invented what is claimed. *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). For example, the Board, quoting the CCPA and Federal Circuit, stated in *Ex parte Sorenson*, 3 USPQ2d 1462, 1463 (BPAI 1987):

> By the same token, we are mindful that appellant's specification need not describe the claimed invention in *ipsis verbis* to comply with the written description requirement. *In re Edwards*, 568 F.2d 1349, 196 USPQ 465 (CCPA 1978). The test is whether the originally filed specification disclosure *reasonably* conveys to a person having ordinary skill that applicant had possession of the subject matter later claimed. *In re Kaslow*, 707 F.2d 1366, 217 USPQ 1089 (Fed. Cir. 1991).

Applicant's specification meets the Section 112, written description requirement for the language "normally transcriptionally silent target gene" because it is clear to one of ordinary skill that Applicant had possession of this subject matter. Applicant asserts that the language "gene of interest may or may not be expressed," when read outside the

E

context of Applicant's specification, may be related to the translational process and unrelated to the transcriptional process. However, such language, when read in the context of Applicant's specification, conveys to one of ordinary skill transcriptional silence. For example, the portion of the specification that discusses the use of homologous recombination to modify the regulatory sequence of a target gene teaches techniques that affect transcription and not translation. See the specification, at page 7, lines 1-35, which discusses such modifications. For example, lines 3-12, of such page state:

> For example, one may wish to change the transcriptional initiation region for the target gene, so that a portion of the homologous region might comprise nucleotides different from the wild-type 5′ region of the target gene. Alternatively, one could provide for insertion of a transcriptional initiation region different from the wild-type initiation region between the wild-type initiation region and the structural gene. (emphasis supplied)

Also, the specification, at page 3, lines 12-17, states:

> Expression of mammalian proteins of interest is achieved by employing homologous recombination for integration of an amplifiable gene and other regulatory sequences in proximity to a gene of interest without interruption of the production of a proper transcript. (emphasis supplied)

These modifications of the transcription initiation sequences and regulatory sequences affect transcription, not translation.

Accordingly, since the portion of the specification that discusses the use of homologous recombination to modify

E

the regulatory sequence of a target gene affects transcription and not translation, the language "gene of interest may or may not be expressed" can only convey to a person of ordinary skill that Applicant had possession of activating a transcriptionally silent gene. Thus, Applicant provides a written description for the language "normally transcriptionally silent target gene."

In addition, the caption for Figure 4 reads, "Targeting of EPO for Gene Activation" (emphasis supplied). This language also provides a written description support for the language "normally transcriptionally silent target gene" because activation clearly means transcribing a silent gene.

Yet also, the example in the specification relating to expressing EPO in human 293 embryonal kidney cells inherently provides a written description for the language "normally transcriptionally silent target gene" because, as discussed in Section 1.1. above, EPO is transcriptionally silent in such cell. One can rely on an inherent feature to meet the written description requirement. *Kennecott Corp. v. Kyocera International Inc.*, 5 USPQ2d 1194 (Fed. Cir. 1987).

Accordingly, Applicant meets the written description requirement for the language "normally transcriptionally silent target gene" and, therefore, requests that this Section 112, first paragraph, objection and rejection be withdrawn.

In addition, Claim 106 satisfies the written description requirement because it covers a human 293 embryonal kidney cell that expresses EPO wherein the genome of the kidney cell has inserted therein the enhancer and promoter

E

of human CMV operatively associated with the EPO gene, which, as acknowledged by the Examiner, is described in the specification.

1.3. The Chappel '071 Patent Is Not Available As Prior Art Under 35 U.S.C. § 102(e)

The rejection of Claim 105 under 35 U.S.C. § 102(e) in view of the Chappel '071 patent is erroneous and should be withdrawn.

The present application is a Rule 60 continuation of application Serial No. 07/787,390 filed November 4, 1991 ("'390 parent application") as the national stage of International application PCT/US90/06436 filed November 6, 1990 ("'436 PCT application"). The disclosure of each application is identical and supports the claims under 35 U.S.C. § 112 as detailed above. Therefore, under 35 U.S.C § 120, the pending claims are entitled at least to the benefit of the November 6, 1990 filing date of the '436 PCT application.[2]

The effective date accorded to the Chappel '071 patent as a reference under 35 U.S.C. § 102(e), as indicated

---

[2] The '390 parent application is also a continuation-in-part of application Serial No. 432,069 filed November 6, 1989 ("'069 grandparent application"). The present application claims benefit of priority under 35 U.S.C. § 120 to each of the '390 parent application, the '436 PCT application, and the '069 grandparent application. The Applicants also contend that the present claims are entitled to the benefit of the November 6, 1989 filing date of the '069 grandparent application, which, but for one example, has an identical disclosure. However, the question of benefit to priority to the '069 grandparent application is irrelevant to any bona fide issue of patentability in this case, since there is no intervening art applied against the claims.

E

on the face of the patent itself, is May 28, 1992 -- almost two years subsequent to the November 6, 1990 filing date which must be accorded to the claims of the instant application. Therefore, the Chappel '071 patent cannot be considered prior art against the claimed invention.[3] Since the Chappel '071 patent purports to claim the same invention as is covered by pending Claims 105 and 106, an interference should be declared to determine who is the first inventor of the conflicting subject matter.

2. NEW CLAIMS 107-118 COVERING METHODS FOR LARGE SCALE PROTEIN PRODUCTION ARE PATENTABLE

Original Claims 26, 38-41 and 69-70 covering mammalian host cells with an integrated regulatory sequence operably associated with an endogenous target gene, and Claims 48, 50, 52-55, and 79 covering methods for producing such cells were rejected under 35 U.S.C. § 103 as obvious over Smithies and Nandi in view of Thompson. Dependent claims specifying: primary host cells (Claims 37, 42, 51 and 56) were rejected further in view of Palmer; mutated target genes (Claims 33-36 and 58-61) were rejected further in view of Frohman and Thomas; and amplifiable genes (Claims 27, 32, 43, 91, 92, 94 and 49, 57, 80 and 81) were rejected further in view of Anderson and Ringold.

Claims 44, 46, 47 and 62, 64-66, 86, 97 and 102 covering secondary expression host cells and methods for their preparation, involving transferring the targeted gene from the

---

[3] The Chappel PCT application, WO91/09955 of record, which corresponds to the Chappel '071 patent, was published July 11, 1991, and likewise is not prior art.

mammalian host cell to a continuous cell line, and methods for their use in protein production were rejected under 35 U.S.C. § 103 as obvious over Smithies, Nandi and Thompson further in view of Nelson. Dependent claims specifying amplifiable genes (Claims 63, 82, 87, 88, 98 and 100) were rejected further in view of Ringold and Anderson.

All dependent claims specifying that the regulatory sequence is a promoter (Claims 73, 74, 77, 78, 84, 85, 95, 96, 103 and 104) were rejected under 35 U.S.C. § 103 as obvious over Smithies, Nandi, and Thompson, and further in view of Nelson or Nelson and Ringold, and further in view of Foecking and Boshart.

2.1. The Bases For The Examiner's Rejections Under 35 U.S.C. § 103

Briefly, the Examiner contends that Thompson provides the motivation to target changes to control sequences, and that Smithies and Nandi provide an expectation of success, thus rendering targeting a gene in accordance with Claims 26, 38-41 and 69-70 obvious. The Examiner further contends that Nelson provides the motivation to transfer chromosomes containing a gene of interest to a secondary host cell, thus rendering Claims 44, 46, 47, 62, 64-66, 86, 97 and 102 obvious. The remaining references are cited to ostensibly support a reasonable expectation of success for targeting primary cells (Palmer), targeting mutations to the target gene (Frohman and Thomas), targeting amplifiable genes (Ringold and Anderson) and targeting the CMV promoter/enhancer (Foecking and Boshart).

The Examiner also contends that the Liskay Declaration should be accorded little weight because, according to the Examiner, unlike the declarant's statements, the claims are not limited to "gene activation" or to "methods for large scale protein production."[4] However, Claims 105 and 106 (submitted subsequent to the Liskay declaration) specify gene activation, and new Claims 107-119 specify methods for large scale protein production. Therefore, the Applicants believe that the foregoing rejections are obviated by the new claims discussed below.

2.2. The Invention Covered By The New Claims

Claims 26, 27, 32-44, 46-66, 69, 70, 73-75, 77-82, 84-88, 91, 92, 94-98, 100, and 102-104 have been cancelled and replaced with new Claims 107-119, which more precisely cover one embodiment of the invention -- large scale protein production in cell culture, involving: targeting an endogenous gene in a mammalian host cell so that an amplifiable gene and/or a regulatory sequence heterologous to the target gene is inserted into the genome in operative association with the target gene; and transferring the recombined target gene to a mammalian continuous cell line which is cultured so that the target gene product is expressed and recovered from the cell culture.

---

[4] The Examiner has characterized Dr. Liskay's statements on the issue of reasonable expectation of success as mere opinion, but has largely ignored the Applicant's and Dr. Liskay's analysis of whether the art relied on suggests the invention. For reasons detailed, infra, the embodiment covered by the new claims are more commensurate in scope with Dr. Liskay's declaration and are not suggested by the references cited.

The claimed invention affords a unique and powerful approach to gene expression, obviating the need to clone the gene itself, and allows for the expression of large genes that are difficult, or impossible to clone. A particular advantage is conferred when the method is applied to the production of human gene products, *e.g.*, as covered by Claims 111 and 114. The improvement afforded by the transfer of the activated/modified recombined target gene to a continuous cell line suitable for large scale culture allows one to produce proteins encoded by genes contained in human target cells that are unsuitable or unusable for long term or large scale culture -- either because the host cell is a transformed human cell line which cannot be approved to manufacture products intended for human use, or because the host cell is a primary cell which is incapable of indefinite proliferation.

In accordance with the invention, genes can be targeted in transformed human cell lines that may be contaminated with viruses, including retroviruses, that are infectious to humans; however, the activated/modified target gene is then transferred to a continuous cell line, such as a CHO (Chinese hamster ovary) cell line that has been approved for production of proteins for human use.

In another aspect of the method of the invention, the use of unsafe cell lines, *e.g.*, transformed human cell lines, can be avoided altogether. For example, homologous recombination can be used to target an amplifiable gene and/or a regulatory sequence in operative association with a target gene in the genome of a <u>normal</u>, <u>non-transformed</u>, <u>primary</u> <u>human cell</u> (*e.g.*, as covered by Claims 112 and 113); production of

the gene product is achieved when the recombined target gene is transferred to a continuous cell line that can be used for large-scale culture, and which has been approved for production of gene products for human use. As a result, the process and product obtained are safe for human use.

The art relied on by the Examiner does not address this issue, much less provide the solution afforded by the claimed method. Moreover, the steps of the method, which are more precisely covered by the new claims, are not suggested by the art of record. Therefore, the Examiner's rejections of record are obviated, and new Claims 107-119 should be allowed. However, in order to be completely responsive to the Examiner's rejections, Applicant's reasoning is set forth in detail below.

2.3. Thompson, Smithies, Nandi, And Nelson Whether Considered Individually Or In Combination Do Not Suggest The Claimed Invention

The combination of Thompson with Smithies and Nandi, with or without Nelson, does not suggest the steps of the method covered by the new claims: targeting a regulatory sequence (such as an exogenous promoter), with or without an amplifiable gene, into a mammalian host cell so that the inserted elements are operatively associated with an endogenous target gene, and the subsequent transfer of the recombined target gene from the mammalian target cell to a continuous mammalian cell line for large scale production of the target gene product in culture. Therefore, the invention, as claimed, cannot be obvious.

2.3.1. The Legal Standard To Establish A *Prima Facie* Case of Obviousness

First, the Federal Circuit *In re Vaeck*, 20 USPQ2d 1438, 1441 (Fed. Cir. 1991) has stated that a proper Section 103 analysis requires, *inter alia*:

> (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. See *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. Id.

Second, the Federal Circuit has stated time and again that it is impermissible within the framework of a Section 103 rejection to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary for the full appreciation of what the reference fairly suggests to one of ordinary skill in the art. One cannot consider a reference in less than the entirety, *i.e.*, disregard disclosures in the reference that diverge from and teach away from the invention. Specifically, the Federal Circuit, in *In re Hedges*, 228 USPQ 685, 687 (Fed. Cir. 1986) stated:

> We agree with Hedges that the prior art as a whole must be considered. The teachings are to be viewed as they would have been viewed by one of ordinary skill. *Kimberly-Clark v. Johnson & Johnson*, 745 F.2d 1437, 1454, 223 USPQ 603, 614 (Fed. Cir. 1984); *In re Mercier*, 515 F.2d 161, 1165, 185 USPQ 774, 778 (CCPA 1975). "It

> is impermissible within the framework of a Section 103 rejection to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what the reference fairly suggests to one of ordinary skill in the art". *In re Wesslau*, 353 F.2d at 241, 147 USPQ at 393.

The Federal Circuit in *W.L. Gore & Associates v. Garlock, Inc.*, 220 USPQ 303 (Fed. Cir. 1983), in regards to the obviousness issue, recited, at page 311, "the district court erred . . . in considering the references in less than their entireties, *i.e.*, in disregarding disclosures in the references that diverge from and teach away form the invention at hand." (emphasis added). The Federal Circuit went on to state:

> To imbue one of ordinary skill in the art with knowledge of the invention in suit when no prior art references of record convey or suggest that knowledge is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher. It is difficult but necessary that the decision maker forget what he or she has been taught at trial about the claimed invention and cast the mind back to the time the invention was made (often as here many years), to occupy the mind of one skilled in the art who is presented only with the references, and who is normally guided by the then-accepted wisdom in the art (emphasis added).

Id. at page 313.

Finally, the Federal Circuit has also stated time and again that for the disclosures of two or more prior art references to be combined in order to establish *prima facie* obviousness "[t]here must be some suggestion for doing so, found either in the references themselves or in the knowledge

generally available to one of ordinary skill in the art." *In re Jones*, 21 USPQ2d 1941, 1943-1944 (Fed. Cir. 1992); *In re Fine*, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988).

2.3.2. The Teachings Of Thompson, Smithies, Nandi and Nelson

Briefly, Thompson relates to targeting modifications to genes contained in an animal's genome and transmitting the modified gene to the animal's offspring. Thompson describes using homologous recombination in a mouse ES (embryonic stem) cell line,[5] and introducing the modified ES cell line into blastocysts in order to transmit the modified gene to offspring. In Thompson's approach, the "target" gene is native to the ES cell line -- the targeted recombined gene is never removed and transferred to a different host for expression and production in cell culture -- a clear requirement of the invention now claimed.

Also, Thompson does not suggest the replacement of the endogenous promoter with a strong promoter, but instead refers only to unspecified "changes" to control sequences that could be useful for the study of gene expression and mapping promoters. At most, Thompson speculates, at page 313, that such techniques may potentially be used in livestock animals "to increase output, or produce novel materials." Thompson's speculation that livestock animals potentially might be engineered to "increase output," *i.e.*, make larger cows or sheep or increase milk or wool production, does not even

---

[5] Thompson uses an HPRT-deficient ES cell line referred to as E14TG2a.

relate to the subject matter of the invention, *i.e.*, large-scale production and recovery of a protein, much less how to accomplish that in cell culture as specified by new Claims 107-119. Thompson's further speculation that livestock animals might be engineered to produce "novel materials", relates to the insertion of foreign genes into the genome of the animal; this also has nothing to do with the method of the invention specified by the claims, where regulatory sequences are targeted to a native gene for large scale production of a protein in cell culture.

Accordingly, Thompson, when read in its entirety as required by *Hedges*, *supra*, and *W.L. Gore*, *supra*, does not teach or suggest large scale protein production in cell culture, *i.e.*, the goal of the invention, and how to accomplish it, *i.e.*, by the targeting and transfer steps delineated in the pending claims.

The motivation missing from Thompson is not supplied by Smithies, Nandi or Nelson.[6] Smithies describes the use of homologous recombination to insert a test plasmid into the β-globin locus of a hybrid cell line.[7] Smithies does not suggest the targeted replacement of a native promoter and/or the transfer of the targeted gene to a secondary host cell for

---

[6] Even the Examiner must agree that Smithies and Nandi do not supply the motivation missing from Thompson -- the Examiner has not cited Smithies and Nandi for this proposition. Instead, the Examiner relies on Smithies and Nandi for allegedly supporting a reasonable expectation of successfully accomplishing targeted homologous recombination in all cell types.

[7] Smithies describes the use of a hybrid cell line made by fusing mouse erythroleukemia cells to human fibroblasts, referred to as the Hull hybrid.

E

large scale production of the gene product -- Smithies does not relate to large scale production of any gene product. Instead, Smithies at best suggests that targeted modifications may eventually be useful for gene therapy -- *i.e.*, to correct defective genes in patients with hemoglobinopathies, to treat such diseases as thalassemia and sickle-cell anemia. Acknowledging that this long term goal may not be practical, Smithies indicates that extensions of the principles used should permit "new approaches to many problems in molecular genetics." However, this is a far cry from a suggestion of the specific method of the invention as presently claimed, as required by *Vaeck*, *supra*, and, therefore, could not possibly render the invention obvious.

Nandi describes the use of homologous recombination to insert cloned genes at specific loci in the genome of the same hybrid cell line described in Smithies, in order to study the effect of the site of integration on the expression of the inserted cloned gene.[8] Nandi does not describe or suggest the targeted replacement of the native promoter of an endogenous target gene -- quite the opposite. Nandi describes the integration of a foreign cloned gene into a host cell genome

---

[8] The Examiner is incorrect when she states that Nandi discloses that the expression of an endogenous target gene was controlled by integrated regulatory elements (see unauthorized Final Office Action, paper No. 18, mailed March 10, 1995 at pp. 16, 25, and 26, which refer to Nandi, p. 3848 and Fig. 1 and 1b). The text and legend to this figure show that Nandi was assaying for expression of the inserted foreign gene ($\Delta\beta$) and noted that the inserted foreign gene reduced expression of neighboring resident genes, probably due to disruption of the neighboring gene. There is no teaching suggestion or evidence in Nandi that expression of any resident gene was controlled by the promoter of the inserted foreign gene.

so that expression of the foreign gene is controlled by its own promoter and influenced by an endogenous host cell enhancer -- the exact opposite of the claimed invention. In fact, Nandi reports that integration of the foreign cloned gene reduces expression of neighboring endogenous genes, perhaps due to disruption (see Nandi, at p. 3848 "Expression of the resident β-globin gene in the modified locus" at col. 1 bridging over to col. 2) -- again, the opposite of the claimed method of the invention. In addition, Nandi suggests isolating promoters from the β-globin locus so that they can be used in standard expression vectors to achieve controlled expression of genes integrated at random sites in the genome (Nandi, p. 3849, col. 1, last ¶) -- an approach which avoids targeted modifications altogether. Finally, Nandi also does not suggest the transfer of a targeted gene to an expression host. It is clear that Nandi does not relate to large scale protein production of any gene product, but rather to the use of recombinant DNA techniques, targeted or standard, for gene therapy purposes.

Nelson does not relate to methods for gene expression, gene targeting or large scale protein production in cell culture. Moreover, contrary to the Examiner's assertion, when Nelson is read for what it fairly suggests to one of ordinary skill, Nelson does not provide the motivation to combine the references relied on by the Examiner. Instead, Nelson describes a method for transferring metaphase chromosomes from one cell to another in order to generate somatic cell hybrids useful for chromosome mapping. Nelson's technique involves the use of retroviruses to randomly

integrate a selectable marker into the genome of donor murine cells -- no targeting at all is used or suggested. After transfer of the murine metaphase chromosomes to hamster or monkey cells, the recipient cells are grown under selective pressure to select those hybrid cells that acquired a transferred chromosome segment containing the selectable marker. The organization of the transferred DNA in the hybrid cells was analyzed by Southern transfer hybridization -- expression of transferred donor cell genes was not engineered, sought or evaluated.

Nelson's technique relates to gene mapping and the isolation and cloning of genes. Nelson purports to allow the study and mapping of chromosome regions previously refractory to analysis by chromosome transfer techniques. Accordingly, when one reads Nelson for what it fairly suggests to one of ordinary skill, as required by *Hedges, supra*, and *W.L. Gore, supra*, *Nelson* is simply not directed to expression of gene products for large scale protein production. Thus, how can Nelson possibly suggest transferring a target gene to a cell line for large scale protein production? Clearly, this constitutes hindsight, which is not permitted as recited in *W.L. Gore, supra*.

2.3.3. The Combined Teachings Of Thompson, Smithies, Nandi and Nelson Do Not Relate To Large Scale Protein Production In Cell Culture

There is no basis to combine the teachings of Thompson, Smithies, Nandi and Nelson, as required by *Jones, supra*. Thompson relates to the production of genetically altered animals. Smithies and Nandi relate to targeting

E

corrections into genes for gene therapy. Nelson relates to techniques for preparing hybrid cells used for gene mapping. Thus, Thompson, Smithies, Nandi and Nelson are such disparate teachings, it is hard to imagine how the references would properly be combined, as required by *Jones*, *supra*, to support an obviousness rejection of the claimed method -- a method which relates to an entirely different goal, *i.e.*, large-scale protein production in cell culture using a unique method involving targeting and transfer.

Furthermore, even when considered in combination, Thompson, Smithies, Nandi and Nelson do not relate to strategies that are useful for large-scale protein production in mammalian cell culture systems -- much less the method specified in the new claims!

Instead, Thompson describes the use of homologous recombination in mouse ES cell lines to introduce targeted modifications into the mouse genome, and the transmission of the modified gene to offspring. At most, Thompson speculates that such techniques may be applied to livestock animals to increase their output (*e.g.*, meat, milk, wool) or to produce novel, foreign gene products in the animals -- not large scale protein production in cell culture. Smithies and Nandi do not cure the deficiency. Smithies describes the use of homologous recombination to target modifications into the β-globin locus of a hybrid cell line, and suggests that such techniques may someday be useful for treating patients with hemoglobinopathies, *i.e.*, gene therapy. Nandi describes the use of homologous recombination to introduce cloned genes into the β-globin locus in the hybrid cell line used by Smithies in

order to study the effects of the site of integration on the expression of the inserted foreign cloned gene.

Nelson, also, has nothing to do with either targeted homologous recombination or gene expression let alone large scale protein production. Instead, Nelson describes DNA transfer techniques useful for generating hybrid cells for gene mapping. This has nothing to do with transferring target genes for large-scale protein production.

Thus, the references even when considered in combination, do not relate to or address large-scale production of proteins in culture by any technique, let alone the Applicant's claimed method!

2.3.4. The Combined Teachings Of Thompson, Smithies, Nandi and Nelson Do Not Suggest The Steps Of The Claimed Method: Targeting Followed By Gene Transfer To Achieve Protein Production In Cell Culture

The combined teachings of Thompson, Smithies, Nandi and Nelson do not suggest or arrive at the Applicant's unique approach for large-scale protein production in cell culture. None of the references, whether considered individually or in combination, suggest the targeted introduction of an amplifiable gene and/or regulatory sequence heterologous to a mammalian target gene in a mammalian host cell, and the subsequent transfer of the recombined targeted gene to a mammalian continuous cell line suitable for large-scale culture, so that high levels of the target gene product are expressed and recovered from the mammalian cell culture.

Thompson's work relates to targeting modifications to genes contained in an animal's genome, by targeting a gene

E

contained in an ES cell line, and introducing the modified ES cell line into blastocysts in order to transmit the modified gene to offspring. Smithies and Nandi relate to the introduction of foreign cloned genes into site specific locations in the β-globin locus of the genome of a hybrid cell line. In Thompson, Smithies and Nandi, the modified DNA is never removed and transferred to a different host cell for any type of expression.

The only reference involving DNA transfer, Nelson, does not relate to expression of a gene of interest at all -- Nelson is concerned with transferring the genome of one cell into a recipient cell in order to create a hybrid cell used for gene mapping. Nelson does not suggest altering the transferred DNA by targeting or otherwise, or engineering the transferred DNA in order to obtain any expression much less the high level expression of a gene of interest required for large-scale protein production in culture.

2.3.5. The Combined Teachings Of Thompson, Smithies, Nandi and Nelson Do Not Suggest Targeted Replacement Of The Target Gene's Native Promoter

Thompson, Smithies, Nandi and Nelson, whether considered individually or in combination, do not suggest the wholesale replacement of the native promoter of a mammalian target gene, *i.e.*, the targeted introduction of a strong exogenous promoter, heterologous to the target gene, that is capable of driving high level expression of the target gene in mammalian cells in culture. In the experiments and data reported, Thompson describes the correction of a deletion mutation by restoring the missing parts of the deletion

mutant, including the native promoter, to the Hprt gene in the mouse genome, and transmitting the corrected gene to offspring. Thompson's speculation that targeted homologous recombination would make it possible to target unspecified "changes" to the control sequences of genes in the animal falls short of the mark -- it does not suggest "knocking out" the native promoter and the targeted introduction of a strong exogenous promoter for driving high level expression required for large-scale protein production in cell culture.

Indeed, in Thompson's ES system, such a change could have deleterious consequences to the animal generated. For example, if a regulated gene were subjected to the control of a strong constitutive promoter, the result of such continuous, unregulated high level expression in the resulting offspring could be deleterious, and lethal to the animals generated.

Moreover, when taken in context with the entire article, it appears that Thompson's "changes" to the control sequences merely refer to targeting mutations to the native promoter in order to "map" the promoter; *i.e.*, to determine the mechanism by which expression of the Hprt gene is elevated in brain tissue (see Thompson at p. 319, col. 2, lines 55-58). Given the constraints of Thompson's system, and considering the isolated statement relied on by the Examiner in the context of the entire article, Thompson's speculation concerning unspecified "changes" to the control sequences would not be interpreted by one skilled in the art to mean the wholesale replacement of the endogenous, native promoter for an exogenous, strong promoter, suitable for driving high level expression in large-scale protein production cell culture.

Again, the deficiency in Thompson is not cured by Smithies, Nandi and/or Nelson. Both Smithies and Nandi involve the insertion of a foreign cloned gene into the β-globin locus of a hybrid cell line. Nandi notes that expression of cloned genes containing the foreign insert was influenced by endogenous promoters and enhancers -- an arrangement of elements opposite to that of the presently claimed invention -- and that the insertion of the foreign cloned gene reduced expression of neighboring endogenous genes, probably due to disruption of the neighboring endogenous genes -- again, an arrangement of elements opposite to that of the claimed invention. Nelson, a gene mapping technique, does not describe or suggest modifying the transferred DNA at all -- much less in a targeted manner. Instead, Nelson describes the retroviral-mediated integration of a selectable marker randomly into the donor genome to increase transfer efficiency in the recipient cell to improve gene mapping. Expression is not engineered, sought or evaluated in Nelson's technique.

In view of the foregoing, the invention covered by the amended claims is not suggested by Thompson, Smithies, Nandi, and Nelson, whether considered individually or in combination. Therefore, the claimed invention is not obvious.

2.4. The Secondary References Do Not Cure The Deficiencies Of Thompson, Smithies, Nandi and Nelson

The suggestion lacking in the combination of Thompson, Smithies, Nandi and Nelson is not supplied by

Palmer, Frohman and Thomas, Ringold and Anderson, or Foecking and Boshart. Therefore, the invention is not obvious.

Palmer describes the retroviral mediated transfer of cloned genes into fibroblasts for use as vehicles in gene therapy. In particular, Palmer describes the transfer of the cloned ADA (adenosine deaminase) gene into ADA-deficient fibroblasts and suggests that such fibroblasts could be used in patients, *e.g.*, as part of a skin graft, to deliver the ADA gene product *in vivo*. Even when combined with Thompson, Smithies, Nandi and Nelson, there is no suggestion of how primary cells such as fibroblasts could be used for large-scale protein production in cell culture. Certainly, there is no hint that primary cells, such as fibroblasts, could be used in the method of the invention as set forth, for example in Claims 112 and 113; *i.e.*, by targeting an amplifiable gene and/or exogenous regulatory sequence into a fibroblast genome and transferring the recombined target gene to an expression cell line for large scale protein production in culture.

Thomas and Frohman describe the uses of homologous recombination to alter genes in animals such as mice by targeting charges to the genes in ES cell lines which are then incorporated into blastocysts so that the altered gene is transmitted to offspring. Thomas describes the use of targeted homologous recombination to disrupt a resident gene and knock out its expression. Frohman is a review article describing the work of Smithies and Thomas. These studies, which relate to gene transmission in animals and knock-outs add nothing to the teachings of Thompson, Smithies, Nandi and Nelson to suggest the invention as claimed, *i.e.*, that

targeted homologous recombination should be used to target amplifiable genes and/or exogenous regulatory sequences into a mammalian host cell genome, and that the recombined target gene should be transferred to an expression host cell for large scale protein production in cell culture.

Ringold and Anderson describe the use of amplifiable genes for standard cloning schemes and for gene therapy, whereas Foecking and Boshart describe the CMV promoter/enhancer which can be used in the construction of expression vectors. However, there is no suggestion that such genes and/or regulatory elements could or should be engineered into target cells using the method covered by the pending claims (*e.g.*, Claims 116-118); *i.e.*, by targeting such sequences into a mammalian host cell to activate or enhance the expression of a target gene endogenous to the host cell, and transferring the recombined target gene to an expression host cell for large scale protein production in culture.

In view of the foregoing, the art relied on by the Examiner does not render obvious the method of the claimed invention.

2.5. The Rejections Under 35 U.S.C. § 112 Are Obviated And/Or Overcome

The Examiner's rejections under 35 U.S.C. § 112, first and second paragraphs, are obviated by the new claims, which adopt the Examiner's suggestion, *e.g.*, to recite integration within an intron, and not the phrases objected to by the Examiner, *e.g.*, "so that the endogenous target gene is

not disrupted."[9] However, the Applicant knows of no support for the Examiner's position on p. 4 of the April 25, 1995 Office Action that would require the amplifiable gene to be positioned 5' to the target gene in order to result in amplification under selection pressure. In this regard, the Examiner's attention is invited to "Molecular Cloning. A Laboratory Manual, 2d Edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press at 16.9-16.10 and 16.28-16.29 a copy of which is submitted herewith. The Cloning Manual explains that amplifiable genes and adjoining DNA sequences (whether 5' or 3') are amplified in response to selective pressure. In fact, when used in connection with standard recombinant DNA cloning technology, the amplifiable gene and cloned gene used to transform host cells do not have to be carried on the same DNA molecule! How then, could the Examiner assert that it would require undue experimentation to determine whether the amplifiable gene would function when inserted 3' to the target gene? Also, the specification does, in fact, describe the integration of the amplifiable gene "within the vicinity of" or "adjacent to" the target gene and such written description is not confined to the 5' flanking sequence of the target gene. (Specification, p. 4, lines 5-13; p. 5, lines 11-14). Indeed, the specification describes

---

[9] The Applicant disagrees with the Examiner's analysis that such term is new matter (p. 3 of the April 25, 1995 Office Action). Again, the Examiner is simply reading words out of context, and is not evaluating the words in the context of the invention described in the specification. However, to narrow the issues and obviate the rejection, the phrase is not used in the new claims.

targeting either or both the 5' or 3' flanking regions of the target gene (specification at p. 6, lines 1-38).

In view of the foregoing, all rejections under 35 U.S.C. § 112, first and second paragraphs, should be withdrawn.

CONCLUSION

Applicant respectfully requests entry and consideration of the foregoing amendments and remarks. The amended claims are believe to be free of the art, and patentable. Withdrawal of all rejections and objections. An early allowance is earnestly requested.

Respectfully submitted,

Date: Oct 25, 1995

Laura A. Coruzzi 30,742
LAURA A. CORUZZI (Reg. No.)

PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

105. A mammalian host cell that expresses a gene product encoded by a ~~normally transcriptionally silent~~ target gene within the genome of the host cell, said genome having inserted therein a heterologous regulatory sequence operatively associated with the normally ~~transcriptionally silent~~ target gene, so that the mammalian host cell expresses the target gene product.